CLAIMS

What is claimed:

1. A method for imaging tissue, comprising the steps of:

mounting the tissue on a computer controlled stage of a microscope;

determining volumetric imaging parameters;

directing at least two photons onto a region of interest;

scanning the region of interest across a portion of the tissue;

imaging a plurality of layers of the tissue in a plurality of volumes of the tissue in the region of interest;

sectioning the portion of the tissue and imaging a second plurality of layers of the tissue in a second plurality of volume of the tissue in the region of interest;

detecting an image of the tissue due to said excitation light; and

processing three-dimensional data that is imaged to create a three-dimensional image of the region of interest.

2. The method of Claim 1, wherein the microscope comprises a multi-photon microscope.

3. The method of Claim 1, wherein the detected image is a fluorescent image.

4. The method of Claim 1, wherein the image is a confocal reflectance image.

5. The method of Claim 2, wherein the penetration depth of the multi-photon microscope is in the range of approximately 200-500 μm.

6. The method of Claim 1, wherein the step of sectioning further comprises a microtome system integral with the microscope.

7. The method of Claim 1, wherein the speed of the step of imaging comprises at least 5 frames per second.

8. The method of Claim 1, wherein the step of scanning further comprises video rate scanning.

9. The method of Claim 1, further comprising providing a depth resolution of approximately 0.1 to 2 μm.

10. The method of Claim 1, wherein the step of scanning further comprises a low resolution mode and a high resolution mode.

11. A system for providing a three-dimensional image of a region of interest, comprising:

a light source for producing excitation light and providing at least two photons onto the region of interest;

a scanning microscope optically coupled to the light source;

a tissue sectioning device mechanically coupled to the microscope;

an x-y scanner to scan the region of interest;

an image sensor that detects a plurality of images of the region of interest; and

a data processor that processes the plurality of images to produce a processed three-dimensional image of the region of interest.

12. The system of Claim 11, wherein the microscope comprises a multi-photon microscope.

13. The system of Claim 11, wherein the microscope comprises a confocal microscope.

14. The system of Claim 11, wherein the light source is a Titanium-Sapphire laser.

15. The system of Claim 11, wherein the light source is one of a picosecond laser and femtosecond laser.

16. The system of Claim 11, further comprising a rotating polygonal mirror that provides a fast scanning axis.

17. The system of Claim 11, further comprising a galvanometer driven mirror that provides a slow scanning axis.

18. The system of Claim 11, further comprising a piezoelectric-driven lens translator that provides a depth axis.

19. The system of Claim 11, further comprising at least one diode to generate a reference signal.

20. The system of Claim 11, wherein the image sensor is one of a charge coupled device, photomultiplier tube and avalanche photodiodes.

21. The system of Claim 11, wherein the excitation light is in the range of approximately 650-1200 nm.

22. The system of Claim 11, wherein the tissue sectioning device is one of a microtome, a vibratome and a rotating blade.

23. The system of Claim 11, further comprising a low resolution scanning mode and a high resolution mode for focusing in on a region of interest.

24. A method of imaging tissue in-vivo, comprising the steps of:

mounting the tissue in a multi-photon microscope;

directing at least two photons onto a region of interest;

scanning a plurality of layers of the tissue in the region of interest;

imaging a plurality of layers in the tissue in the region of interest;

detecting a fluorescence image of the region of interest due to said excitation light; and

processing the detected fluorescence image comprising the steps of:

sequentially storing a plurality of portions of three-dimensional image data set;

enhancing the image data set;

registering individual three-dimensional data sets to generate a large three-dimensional data set; and

displaying the three-dimensional data set of the region of interest.

25. The method of Claim 24, wherein the step of processing further comprises compressing the three-dimensional data set.

26. The method of Claim 24, wherein the step of processing further comprises identifying and quantifying features of the region of interest.

27. The method of Claim 24, wherein the step of processing further comprises analyzing the three-dimensional data set.

28. The method of Claim 24, wherein the step of imaging further comprises imaging mitotic recombination in the tissue.

29. The method of Claim 24, wherein the step of scanning further comprises a low resolution mode and a high resolution mode.